REMARKS

In the Final Action, Claims 1-85 are pending. Claims 8-27 and 39-68 are under examination. Claims 1-7, 28-38 and 69-85 have been withdrawn from consideration as a result of the restriction requirement. The Examiner states that claims 51, 56 and 60-63 would be allowable if these claims are rewritten in independent form to include all of the limitations of the base claim (i.e., Claim 50) and any intervening claims. Claim 14 has been rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enabling support. Claims 8-13, 15-27, 47-50, 52-54, 58 have been rejected under 35 U.S.C. 102(b), as allegedly anticipated by Flax et al. (*Nat. Biotech* (1998) 16: 1033-39) ("Flax et al."). Claims 39-49 have been rejected under 35 U.S.C. 102(b), as allegedly anticipated by Shamblott et al. (PNAS, 95: 13726-31) ("Shamblott et al."). Claim 39 has also been rejected under 35 U.S.C. § 112, second paragraph, as allegedly unclear. Claims 8-13, 15-27, 50-54 and 58 have been rejected under 35 U.S.C. 102(b) as allegedly anticipated by Vescovi et al. (Exp. Neurol 156: 71-83, March 1999) ("Vescovi et al."). Claims 8, 10-13, 15, 16, 23-25, 50, 52-55, 58 and 59 have been rejected under 35 U.S.C. 102(b) as allegedly anticipated by Anderson et al. (U.S. Patent No. 5,693,482, published December 2, 1997) ("Anderson et al."). Claims 8, 11-13, 15, 16, 23-25, 50-54 and 58 have been rejected under 35 U.S.C. 102(a) as allegedly anticipated by Johansson et al. (Exp. Cell Res. 253: 733-736, Dec. 1999) ("Johansson et al."). Claims 64-68 have been rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Thomson in view of Johansson et al.

This Response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Specifically, Applicants have canceled claims 1-7, 28-38 and 69-85, which have been withdrawn from consideration as a result of the restriction requirement. Applicants reserve the right to pursue the subject matter of these claims in one or more divisional applications.

Furthermore, Applicants have canceled claims 8-27, 47-50, 52-55, 59 and 69-85, rendering the rejection of these claims moot. Applicants reserve the right to pursue the subject matter of these claims in a continuation application.

Applicants have also amended claims 51, 56 and 60-63, which are indicated as allowable in the Final Action, to incorporate all of the limitations of the base claim (i.e., claim 50) and any intervening claims. Furthermore, Applicants recognize that claim 57, which depends on allowable claim 56, is not under any rejection in the Final Action and is presumably allowable as well. Applicants have also amended claim 58 to delete the reference to claim 55 and to depend from claims 56 and 57 instead. Finally, Applicants have also added claims 86-87, which depend on claims 58 and 63, respectively. Support for claims 86-87 is found in original claims 58 and 63, respectively. Applicants respectfully submit that claims 51, 56-58, 60-63 and 86-87 are in condition for allowance.

Applicants now address the outstanding rejections with respect to the remaining claims, i.e., claims 39-46 and claims 64-48. In the first instance, claim 39 has been rejected under 35 U.S.C. §112, second paragraph, as allegedly unclear for the recitation "somatic differentiation". Specifically, the Examiner alleges that the specification fails to provide a definition for somatic differentiation. For example, the Examiner questions whether the cells are only capable of differentiating into somatic cells; and if so, what conditions would provide such controlled differentiation. The Examiner has also rejected claims dependent on claim 39 (claims 40-46) as unclear.

It is respectfully submitted that the term "somatic differentiation" is well understood by those skilled in the art. As defined in the New Shorter Oxford Dictionary (1993 edition) and the Encyclopaedia of Molecular Biology (Kendrew) 1994 edition, the term somatic cell means "body cell". Hence, the term "somatic differentiation" means differentiation towards cells of the body. Whereas it is known that embryonic stem cells have the potential to develop into both somatic cells as well as extraembryonic cells, the presently claimed methods induce differentiation of embryonic stem cells towards the somatic lineage, as opposed to differentiation

towards the extraembryonic lineage. As to the conditions that could induce controlled differentiation, Applicants respectfully submit that certain specific conditions are set forth in dependent claim 46. As such, it is respectfully submitted that claims 39-46 are not indefinite. Withdrawal of the rejection is therefore respectfully requested.

Claims 39-46 are rejected under 35 U.S.C. §102(b), as allegedly anticipated by Shamblott et al. (PNAS, 95: 13726-31) ("Shamblott et al.").

According to the Examiner, Shamblott et al. teach the generation of pluripotent human ES cells from cultured human primordial germ cells ("PGCS"). Gondal ridges from post-fertilization human embryos were collected and the cells cultured. The cells were found to test positive for five immunological markers of ES cells. The immunohistochemical analysis of embryoid bodies collected from a culture of the cells revealed a wide variety of differentiated cell types, including derivatives of all three embryonic germ layers. Particularly, the immunohistochemical analysis of the embryoid bodies found ectodermal derivatives of cells suggestive of neuroepithelia and antineurofilament cells. Shamblott et al. therefore conclude that the PGCS are pluripotent stem cells that are positive for markers commonly used to identify pluripotent stem cells, have morphology similar to mouse ES and EG cells, maintain a normal and stable karyotype, and can be differentiated into a wide variety of cell types.

In response to the Examiner's contentions, Applicants respectfully submit that the present claims are directed to a method of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells by providing a controlled differentiating condition, which is non-permissive for stem cell renewal, does not kill cells or induce unidirectional differentiation toward extra-embryonic lineages. Applicants respectfully submit that Shamblott et al. do not teach a controlled differentiating condition as characterized in the present claims.

More specifically, Shamblott et al. do not teach culturing the cells at either a high density, for a long period of time, or in serum free media, as recited in instant claim 46.

Additionally, Shamblott et al. do not teach culturing the cells on a fibroblast feeder layer that does not kill cells or induce unidirectional differentiation toward extra-embryonic

lineages, as also recited in claim 46. In particular, Shamblott et al. do not recognize that there are subsets of fibroblast feeder layers which would support controlled differentiation of the embryonic stem cells. In contrast, as described in the present specification on page 28, line 23 to page 29, line 12, the preparation, handling and testing of fibroblasts are important to the success of the claimed method. Some strains or batches of fibroblasts are more suitable for the maintenance and the induction of somatic differentiation than other strains or batches. Once a batch of fibroblast feeder cells is identified, such batch can be stored and resurrected for subsequent use in directing the induction of somatic differentiation.

Furthermore, Shamblott et al. do not teach culturing the cells on semi-permeable membranes so as to create structures mimicking the postimplantation phase of human development, recited in instant claim 46. Moreover, Shamblott et al. do not teach culturing the cells in the presence of a chemical differentiation factor selected from the group including bone morphogenic protein-2 or antagonists thereof.

The Applicants further observe that Shamblott et al. allegedly identified neural cell types in Embryoid Bodies (EB's), which were generated only with the addition of hrLIF to the cell culture. See page 13729, left column, first full paragraph. In contrast, according to the present application, e.g., at pages 64-65, LIF is not required for the induction of somatic differentiation of human embryonic stem cells.

Moreover, Applicants observe that the pluripotent embryonic stem cells employed by Shamblott et al. are obtained from gonadal ridges and mesenteries of 5-9 week postfertilization human embryos. Shamblott et al. do not teach or suggest culture conditions that would induce somatic differentiation of pluripotent embryonic stem cells prepared from inner cell mass (ICM), as characterized in instant claims 46 and 47.

Accordingly, it is respectfully submitted that Shamblott et al. do not teach the claimed methods. Withdrawal of the rejection under 35 U.S.C. §102(b) based on Shamblott et al. is respectfully requested.

Claims 64-68 have been rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Thomson in view of Johansson et al.

According to the Examiner, Thomson teaches the isolation of primate embryonic stem cells that are characterized by the cell surface markers. Thomson also allegedly teaches methods of isolating a primate blastocyst, isolating cells from the inner cell mass ICM of the blastocyst, plating the ICM cells on a fibroblast layer layer. The Examiner alleges that Thomson further teaches that by manipulation of culture conditions, the primate ES cells can be induced to differentiate into specific cell types, such as neuron cells. The Examiner also alleges that Thomson teaches that the when grown on embryonic fibroblasts and allowed to grow for two weeks after achieving confluence, primate ES cells will spontaneously differentiate.

The Examiner admits that Thomson does not teach culturing neural progenitor cells as spheres or monolayers in serum-free medium comprising DMEM/F12 supplemented with growth factors such as B27, EGF and bFGF. However, the Examiner contends that Johansson et al. teach culturing of human neural stem cells in DMEM/F12 medium, wherein bFGF, B27 supplement and EGF are added. The Examiner also states that Johansson et al. further teach that neurospheres are passed and are dissociated into single cells, which are then picked with a micropipette and transferred to microwells. The Examiner is of the opinion that it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to culture the neural progenitor cells as taught by Thomson in the culturing conditions as taught by Johansson et al., with a reasonable expectation of success.

Applicants first respectfully submit the present methods include obtaining an undifferentiated human embryonic stem cell, and inducing somatic differentiation of the embryonic stem cells to neural progenitor cells by providing differentiating conditions which are non-permissive for stem cell renewal, do not kill cells or induces unidirectional differentiation toward extraembryonic lineages.

Applicants respectfully submit that Thomson does not teach any conditions that would induce somatic differentiation of embryonic stem cells to neural progenitor cells. In this

regard, Applicants observe that the teaching of Thomson at col. 6, lines 9-12, which is relied upon by the Examiner, merely states that ES cells "can be induced to differentiate" into "specific cell types, such as blood cells, neuron cells ..." by "manipulating culture conditions". This description in Thomson does not set forth any condition, let alone conditions for differentiation of ES cells into neural progenitor cells as presently recited, as opposed to differentiated "neuron cells" described in Thomson. The teaching of Thomson at col. 12, lines 53-59, which is also relied upon by the Examiner, merely discloses spontaneous differentiation of ES cells, which can result in both somatic and extra-embryonic differentiation. In contrast, the claimed methods recite "providing differentiating conditions", which conditions are purposefully provided in a controlled manner to induce somatic differentiation of ES cells into neural progenitor cells. Finally, the Examiner has referred to Thomson at col. 15, lines 30-40. There, Thomson describes the formation of tumors in mice from injected ES cells of a wide range of cell types of al three embryonic germ layers, including neural cells (derivative of ectoderm). However, this disclosure merely indicates the pluripotent potential of ES cells, and is irrelevant to generating neural progenitor cells from ES cells *in vitro*. Therefore, Applicants respectfully submit that the premise of the Examiner's rejection that Thomson teaches neural progenitor cells does not exist in the first instance.

Furthermore, Applicants respectfully submit that it is improper for the Examiner to combine the teachings of Thomson and Johansson et al. and reject the instant claims. In particular, the conditions disclosed by Johansson et al. are directed to maintaining and propagating neural stem cells. According to Johansson et al., the neural stem cells exist in the lateral ventricle wall and the hippocampus of the adult human brain, and can be cultured under conditions disclosed therein. That is, Johansson et al. do not teach the production of neural progenitor cells, which is recited in the presently claimed methods, let alone production of neural progenitor cells by inducing somatic differentiation of embryonic stem cells.

Applicants further respectfully submit that neither Thomson nor Johansson et al. provide those skilled in the art the motivation to apply the conditions of Johansson et al., which

are taught merely as conditions for maintaining and propagating neural stem cells, to embryonic stem cells disclosed by Thomson to induce differentiation of the stem cells into neuroprogenitor cells. Moreover, there is no indication that those skilled in the art would have had a reasonable expectation of success in applying the conditions of Johansson et al. to embryonic stem cells disclosed by Thomson.

Therefore, it is respectfully submitted that the rejection of claims 64-68 under 35 U.S.C. §103(a) based on the combination of Thomson and Johansson et al. is improper. Withdrawal of the rejection is therefore respectfully requested.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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